Trinucleotide repeat polymorphism at the human gamma-B-crystallin gene

M.H.Polymeropoulos, H.Xiao, D.S.Rath and C.R.Merril
National Institute of Mental Health Neuroscience Center, St Elizabeths Hospital, Room 131, 2700 Martin Luther King Avenue, Washington, DC 20032, USA

Source/Description: The polymorphic $(AAC)_n$ repeat begins at base pair 3021 of intron B of the human gamma-B-crystallin gene on chromosome 2q34−35 (1). The polymorphism can be typed using the polymerase chain reaction (PCR) as described previously (2). The predicted length of the amplified sequence was 123 bp.

Primer Sequences:
GACAGAGTGAGACTCCATCT (ACC strand);
GATCCTATCTTCTCAGGAGG (TTG strand).

Frequency: Estimated from 50 chromosomes of unrelated individuals. Heterozygosity Index = 68%. PIC = 0.61.

Allele (bp)	Frequency
126	0.30
123	0.36
120	0.02
117	0.32

Mendelian Inheritance: Co-dominant segregation was observed in three CEPH families.

Chromosomal Localization: The human gamma-B-crystallin gene has been assigned to chromosome 2q34−35 (3).

Other Comments: The PCR reaction was performed on 80 ng of genomic DNA using 100 pmoles of each oligonucleotide primer. The samples were processed as described (4) except that the denaturation cycle at 94°C was extended to 1.4 minutes. The trinucleotide repeat was based on a $(AAC)_9$ sequence.

References: 1) Den Dunnen,J.T. *et al.* (1989) *Gene* **78**, 201−213. 2) Weber,J.L. and May,P.E. (1989) *Am. J. Hum. Genet.* **44**, 388−396. 3) Shiloh,Y. *et al.* (1986) *Hum. Genet.* **73**, 17−19. 4) Weber,J.L. *et al.* (1990) *Nucl. Acids Res.* **18**, 4637.

PCR detection of a HindIII polymorphism in the human gene for type II procollagen (COL2A1)

T.Tsuneyoshi, B.P.Sokolov and D.J.Prockop*
Department of Biochemistry and Molecular Biology, Jefferson Institute of Molecular Medicine, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107, USA

The HindIII RFLP in the human gene for type II procollagen (COL2A1) detected by Southern blot analysis (1) proved to be very informative in linkage studies of genetic disorders of connective tissue. Here we report two PCR-based techniques for the rapid detection of the $HindIII^+$ and $HindIII^-$ alleles. The published sequence of the COL2A1 did not identify the polymorphic HindIII site but with a computerized search allowing for one point mutation in the restriction site, four potential HindIII sites within 120 bp region were found (2). Primers 5'-AGAGA-GGAGCGGGCTCAGGA-3' and 5'-CCCCCATGGTTTGCTC-AGTC-3' were used in PCR to amplify this region on a genomic DNA from 20 unrelated individuals.

Polymorphism: Digestion of a 581 bp PCR product with HindIII yielded fragments of 428 and 153 bp if the polymorphic HindIII site was present. This observation demonstrated that the HindIII polymorphism in the COL2A1 gene is a C to T variation in intron 33 in position 1896 of the gene (2). The presence of T yields $HindIII^+$ allele, while C yields $HindIII^-$ allele.

Frequency: Allele frequency for 40 chromosomes of unrelated individuals:
$HindIII^+ = 0.525$
$HindIII^- = 0.475$
Observed heterozygosity = 55%, PIC = 0.374.

Chromosomal Location: COL2A1 has been located at 12q14.3.

Mendelian Inheritance: Co-dominant inheritance has previously been demonstrated (1).

Other Comments: PCR conditions were 1.5 min at 94°C, 1.5 min at 61°C, 2 min at 73°C for 25 cycles. $HindIII^+$ and $HindIII^-$ alleles can also be detected by allele-specific amplification. Primers to amplify the $HindIII^+$-allele were: 5'-ACAGAGAAGTCCCTGCAGTT-3' and 5'-AAGACTCC-TTTCCAAAGCTT-3'; to amplify $HindIII^-$-allele: 5'-ACAGA-GAAGTCCCTGCAGTT-3' and 5'-AAGACTCCTTTCCAAAGCTC-3'. PCR-product size was 395 bp. PCR conditions were 1.5 min at 94°C, 1.5 min at 55°C, 2 min at 73°C for 25 cycles.

Acknowledgements: This work was supported in part by National Institutes of Health Grant AR-39252, a grant from the March of Dimes-Birth Defects Foundation, and a grant from the Markey Foundation.

References: 1) Eug,C.E.L. and Strom,C.M. (1985) *Am. J. Hum. Genet.* **37**, 719−732. 2) Vikkula,M. and Peltonen,L. (1989) *FEBS Lett.* **250**, 171−174.

* To whom correspondence should be addressed